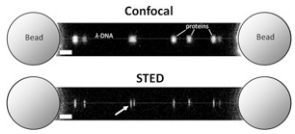
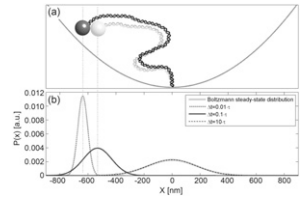
Dense coverage of DNA by proteins is a ubiquitous feature of cellular processes such as DNA replication, transcription, repair, and compaction. We present a single-molecule manipulation and visualization approach capable of studying individual DNA-protein interactions in the presence of a high density of proteins on the DNA and in solution. This approach combines optical tweezers with multicolor confocal fluorescence microscopy and STED nanoscopy. We demonstrate visualization of proteins on DNA with a spatial resolution of 48 nm, about six-fold better than with traditional wide-field microscopy. The resolution enhancement along the direction of the DNA can be seen in the figure below (scale bar 1μm). Two proteins positioned within the diffraction limit can clearly be distinguished in the STED image. In combination with fast confocal line scanning, STED allows real-time imaging of DNA-protein dynamics with a temporal resolution better than 50 ms. The individual trajectories of proteins translocating on DNA can be distinguished at high protein density and tracked with enhanced localization precision. This unique multimodal approach allowed us to visualize, in real time, the assembly of dense nucleoprotein filaments on DNA with unprecedented spatial resolution.



1080-Plat
Routine and Timely Sub-Piconewton Force Stability and Precision for Biological Applications of Atomic Force Microscopy
Allison B. Churnside[1], Ruby May A. Sullan[1], Duc M. Nguyen[1], Matthew S. Bull[1], Gavin M. King[2], **Thomas T. Perkins**[3].
[1]Univ. of Colorado, Boulder, CO, USA, [2]Univ. of Missouri, Columbia, MO, USA, [3]NIST & CU-Boulder, Boulder, CO, USA.
Force drift is a significant, yet unresolved, problem in atomic force microscopy (AFM). We show that the primary source of force drift for a popular class of cantilevers is their gold coating, even though they are coated on both sides to minimize drift. Drift of the zero-force position of the cantilever (z_0) was reduced from 900 nm for gold-coated cantilevers to 70 nm (N =10; rms) for uncoated cantilevers over the first 2 hours after letting the tip; a majority of these uncoated cantilevers (60%) showed significantly less drift (12 nm, rms). Removing the gold also led to ~10-fold reduction in reflected light, yet short-term (0.1-10s) force precision improved. Moreover, improved force precision did not require extended settling; most of the cantilevers tested (9 out of 15) achieved sub-pN force precision (0.54 ± 0.02 pN) over a broad bandwidth (0.01-10 Hz) just 30 min after loading. Finally, this precision was maintained while stretching DNA. Hence, removing gold enables both routine and timely access to sub-pN force precision in liquid over extended periods (100 s). We expect that many current and future applications of AFM can immediately benefit from these improvements in force stability and precision.

1081-Plat
A Hybrid Tirf-Magnetic Tweezers Instrument for Studying Force-Induced Conformational Changes in Proteins
Samuel M. Leachman, Christian A.M. Wilson, Bernardo Cervantes, Athena Ierokomos, Susan Marqusee, Carlos Bustamante.
University of California, Berkeley, Berkeley, CA, USA.
Optical tweezers are a useful research tool for applying forces to single proteins and measuring the resulting changes in extension, but they can only observe conformational changes along the axis of force application and only if the accompanying changes in extension are on the nanometer scale. Our experimental setup skirts this limitation by measuring distance changes using single-molecule Förster resonance energy transfer (smFRET) produced from a total internal reflection fluorescence (TIRF) microscope incorporating magnetic tweezers. Individual protein molecules are conjugated to FRET-paired fluorescent dyes and functionalized DNA handles using disulfide and click chemistry. These handles tether each molecule between a glass coverslip on the TIRF microscope and a paramagnetic bead. An external magnet applies a uniform field that exerts a force on each molecule tethered to the surface. Simultaneous recording of the intensities of the donor and acceptor fluorophores and the magnet position enables direct observation of force-induced conformational changes. The use of triplet-state quenchers and oxygen scavengers extends the lifetime of tethers into the minute timescale, producing single-molecule trajectories exhibiting hundreds of transitions. Here we present our latest results from this new setup.

1082-Plat
A Novel Method for Dynamic Analysis of Single-Molecule Experiments in Trapping Potentials
Yuval Garini[1], Guy Nir[1], Anat Vivante[1], Ian T. Young[2], Moshe Lindner[1].
[1]Bar Ilan University, Ramat Gan, Israel, [2]Delft University of Technology, Delft, Netherlands.
We present a novel method for analyzing the dynamics of tethered particles in single-molecule experiments such as optical and magnetic tweezers, and tethered particle motion.
By using the Smoluchowski equation and its solution, that was not yet fully explored, we developed a method for analyzing the same data being currently measured in the single-molecule experiments mentioned above. We demonstrate the power of the method by analyzing a nano-bead tethered to a single DNA molecule. It allows one to simultaneously extract all the parameters that describe the system, namely, the diffusion coefficient and the restoring-force constant without having to plug-in prior values.
The method is based on the extraction of the moving particle probability distribution function in its complete phase-space. It improves the accuracy of the parameters that are extracted in the experiment, and makes them more robust.



Evolution of the particle distribution function as a function of time, providing the basis for the new analysis method we describe www.igc.ethz.ch and www.gromos.net.

1083-Plat
Detection and Mapping of 5-Methylcytosine and 5-Hydroxymethylcytosine in Short Strands of ssDNA using Nanopore Sequencing with MspA
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University of Washington, Seattle, WA, USA.
Epigenetic modifications of cytosines, such as 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) in CpG sites of DNA, are both, inheritable and influenced by the environment. These nucleotide modifications have been shown to be important in gene regulation, cell programming, and carcinogenesis. It is therefore imperative that next-generation sequencing techniques are able to detect epigenetic modifications. Conventional methods for detection of 5-mCpG and 5-hmCpG, require chemical conversion of 5-mC to uracil or are limited to bulk analysis of relative ratios of C to 5-mC to 5-hmC. Here we demonstrate a technique for mapping individual 5-mCpG and 5-hmCpG sites within single molecules of ssDNA. We used nanopore sequencing whereby the phi29 DNA polymerase draws ssDNA through the porin MspA. An ion current passing through the pore directly detects and maps the location of such modifications in single molecules. We will present data on specific detection of both 5-mCpG and 5-hmCpG sites based on comparisons with unmodified sequences.

1084-Plat
Nanopore Sequencing using a Hidden Markov Model for Base-Calling
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[1]Johns Hopkins University, Baltimore, MD, USA, [2]University of Illinois, Urbana, IL, USA.
Nanopore-based DNA sequencing has many features which recommend it over current state of the art sequencing by synthesis methods; increased read length, reduced sample requirements, and increased speed to name a few. However, the accuracy of base-calling using the electrolytic current has thus far been relatively limited, from both solid-state and biological nanopores. Though part of this is due to the low signal-to-noise ratio, another significant contribution is the base resolution of the nanopore - it does not interrogate a single base at a time, rather the current is influenced by multiple bases at the same time.
We suggest using this multi-base interrogation as an advantage rather than a disadvantage - each base in the DNA strand is read multiple times in this circumstance, allowing for improved accuracy. We have implemented a method to decode the electrical signature of 3bp resolution nanopore electrical measurements into a DNA sequence using a hidden Markov model. We produced simulated ionic current for all 64 possible triplets using atomic-resolution Brownian dynamics (BD).
Using simulated current signatures, we have been able to demonstrate 98.3% base-calling accuracy for λ DNA, a substantially increased value compared to using only a single-base method (47.1%). When applied to 50kb fragments of the human genome, we found a similar median accuracy level (98.2%). Furthermore, we determined that there is a correlation between the local complexity of the sequence, as measured by Shannon's entropy, and the error rate; lower complexity sequence is more error-prone. Longer sequenced fragments have lower error rate - the more information input into the Markov model, the more effective the algorithm is at decoding the DNA sequence; this is in marked contrast to the dephasing problems endemic to current sequencing by synthesis methods.